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ABSTRACT (244/250 words)

The aim of the study was to investigate the effect of static stretching (SS) with different rest intervals on muscle stiffness. Fifteen healthy males participated in the study. Four bouts of thirty-second SS for the gastrocnemii were performed at the maximal dorsiflexion using dynamometer with two different rest intervals between stretches, namely 0 s (R0) and 30 s (R30). Each participant underwent both stretching protocols at least 48 hours apart in a random order. Between each bout of SS, the ankle was moved to 20°-plantar-flexion in 3 s, held for each rest interval time, and then returned to the stretching position in 3 s. The shear elastic modulus of the medial gastrocnemius was measured before (PRE) and immediately after (POST) four bouts of SS to assess muscle stiffness of the medial gastrocnemius. Two-way repeated measures analysis of variance (protocol× time) indicated a significant interaction effect on the shear elastic modulus. The shear elastic modulus significantly decreased after SS in both protocols [R0, PRE: 11.5 ± 3.3 kPa, POST: 10.0 ± 2.6 kPa, amount of change: 1.6 ± 0.9 kPa (13.0 ± 5.2 %); R30, PRE: 11.0 ± 2.8 kPa, POST: 10.2 ± 2.1 kPa, amount of change: 0.8 ± 1.3 kPa (6.0 ± 10.4%)]. Furthermore, the SS with 0-s rest interval induced greater decrease in shear elastic modulus when compared to SS with 30-s rest interval ($p = 0.023$). Thus, when performing SS to decrease muscle stiffness, rest intervals between stretches should be minimized.

1. Introduction

Static stretching (SS) is an effective intervention to decrease the stiffness of a muscle or muscle-tendon unit (MTU) and to improve the joint range of motion (ROM) (Kay et al., 2015; Konrad et al., 2017; Nakamura et al., 2011). Since increased stiffness is considered a risk factor of musculoskeletal injuries, SS is often performed prior to performance to prevent injuries (Herbert et al., 2011; McHugh and Cosgrave, 2010).Therefore, investigating the acute effects of SS is important. With respect to an appropriate SS time to decrease MTU stiffness, a previous study demonstrated that at least 2 min of SS was required to decrease the passive torque of gastrocnemii (Nakamura et al., 2013). In clinical situations, SS is typically divided into multiple repetitions as opposed to being performed continuously for a few minutes (Baechle, 1994), presumably to its ease to perform for therapists and high compliance for patients. When SS is divided into multiple repetitions, it is necessary to consider the total stretching time (i.e., SS time per repetition and number of repetitions) and rest interval time between repetitions. With respect to the total time, a previous study indicated that SS for a constant total time with different time per repetition and number of repetitions (i.e., 60 s × 2 times, 30 s × 4 times, and 10 s × 12 times) causes similar effects corresponding to decreases in stiffness of the gastrocnemii muscles (Nakamura et al., 2017). Conversely, few studies investigated the influence of rest interval time between repetitions. Freitas et al. (2015) compared the improvement in ROM between SS with and without rest interval, and concluded that SS without rest interval was more effective in terms of improving ROM. However, the results of the aforementioned study indicated that the effects of ROM improvement were different based on number of repetitions because the number of repetitions (i.e., total time) was not consistent among participants. Therefore, it is important to investigate the influence of rest interval time between repetitions while holding the total time of SS as a constant.

Increased stiffness of the muscle or MTU has been reported to increase the risk of musculoskeletal injuries (Watsford et al., 2010). It is noted that ROM is insufficient to assess passive mechanical properties because it is also affected by pain and stretch tolerance (Weppler and Magnusson, 2010). A few studies (Halbertsma and Göeken, 1994; Magnusson et al., 1996; McNair et al., 2001) have indicated that the increase in ROM induced by SS might be due only to a change in stretch tolerance without change in passive mechanical properties (so-called 'Sensory Theory' reviewed by Weppler and Magnusson, 2010). Since SS is performed to change not only ROM but also the passive mechanical properties of a muscle or MTU, the effects of SS on passive mechanical properties should be distinguished from stretch tolerance. Hence, passive joint stiffness (which is determined as a slope of torque-angle relationship (Magnusson et al., 1996)) and muscle stiffness (which is represented by shear elastic modulus) are used as indices of stretching effects to assess passive mechanical properties. Passive joint stiffness reflects several factors in addition to muscle stiffness such as the stiffness of joint capsules and ligaments (Maïsetti et al., 2012). Shear elastic modulus measured via ultrasound shear wave elastography (SWE) non-invasively makes it possible to quantitatively assess the muscle stiffness of an individual muscle. Therefore, shear elastic modulus is often used as an index of stretching effect for several skeletal muscles (Ichihashi et al., 2016; Kusano et al., 2017; Xu et al., 2018). Thus, we focused on the stiffness of individual muscles among the passive mechanical properties.

The aim of the present study involves investigating the effect of SS with different rest intervals on muscle stiffness of the medial gastrocnemius. Our hypothesis is that SS with shorter rest intervals leads to a greater decrease in muscle stiffness.

2. Methods

2.1. Participants

Fifteen healthy men (height, 171.4 ± 6.2 cm; mass, 66.7 ± 9.2 kg; age, 24.3 ± 3.0 years) participated in the study. The sample size required for a two-way repeated measures analysis of variance (ANOVA) [effect size = 0.40 (large), α error = 0.05, power = 0.80] was calculated in advance via G*power software (version 3.1.; Heinrich Heine University, Düsseldorf, Germany), and the calculated sample size corresponded to 14. The effect size was determined based on a previous study, which showed the effects of SS on muscle stiffness using two-way analysis of variance (Akagi and Takahashi, 2014). All participants received an explanation about the study and provided written informed consent. The study was approved by the ethics committee of Kyoto University Graduate School and the Faculty of Medicine (R0233-3).

2.2. Experimental protocol

The experimental design was a cross-over design wherein each participant underwent both stretching protocols at least 48 hours apart in a random order. The participants were instructed to maintain their regular physical activities, avoiding unusual exercise between the two sessions. Thirty-second SS for the triceps surae, especially the gastrocnemii in the right leg was repeated for four bouts in the following two protocols: 0-s rest interval (R0) and

30-s rest interval (R30) between each bout of SS. The shear elastic modulus of the medial gastrocnemius (MG) was measured before (PRE) and immediately after (POST) four bouts of SS.

The participants lay prone on a dynamometer (BIODEX System 4, Biodex, USA) with the hip in a neutral position (without any flexion/extension, adduction/abduction, or internal/external rotation) and the knee fully extended, and the foot was attached securely to the footplate of the dynamometer (Fig.1a, b). To define the final angle, the ankle was passively dorsiflexed at 5°/s starting from 30°-plantar-flexion to the maximal dorsiflexion angle (Fig. 1b) that the participants achieved without discomfort or pain (Nakamura et al., 2014). The participants themselves stopped the dynamometer via a remote button. The maximal dorsiflexion angle was defined as the final angle in the study and used for all four bouts of SS.

Surface electromyography (EMG) (TeleMyo2400, Noraxon USA, Scottsdale, AZ, USA) on the lateral gastrocnemius muscle belly was used to ensure that the muscle was inactive during SS and measurements of shear elastic modulus. EMG data was calculated using full-wave rectification and the root-mean-square, with a window interval of 50 ms. Then, the EMG activities during SWE measurements and SS were represented as a percentage of the maximal EMG values during maximal voluntary contraction, which was performed after all other protocols.

2.3. Measurement of shear elastic modulus

The shear elastic modulus of MG was measured to assess muscle stiffness via an ultrasound SWE (Aixplorer, SuperSonic Imagine, France) with a linear probe (4-15 MHz, SuperLinear 15-4, France) in Musculoskeletal (MSK) preset. The measurements were performed in a neutral ankle position (0° plantar-flexion). The measurement site was defined at a level corresponding to proximal 30% of the lower leg length from the popliteal crease to the lateral malleolus in accordance with previous studies (Akagi and Takahashi, 2013; Nakamura et al., 2014). After identifying MG on the ultrasound B-mode image, the measurement site was marked on the skin with a pen to ensure that PRE and POST measurements are performed on the same site. The orientation of the probe was adjusted to the longitudinal plane so that the muscle fascicles were clearly identified on the B-mode image (Hirata et al., 2015; Maïsetti et al., 2012). The region of interest (ROI), 2.25 cm width × 1.75 cm depth, was set near the center of muscle belly bulge of MG, and the image was then obtained. Two images were obtained at each PRE and POST time point. The location of the ROI was kept constant for each participant. The probe was repositioned between capturing two images at each time point. SWE measurements were completed within 1 min. SWE measurements were performed by the same investigator for all participants.

After obtaining the images, a circle with a diameter of 10 mm was drawn at the center of ROI for quantitative analysis (Fig.2). The shear elastic modulus value in the circle was calculated. The shear elastic modulus value (G) is calculated from shear wave speed (V) using the following equation (Gennisson et al., 2010).

$$G\ (\text{kPa}) = \rho V^2$$

where ρ is the muscle mass density (1000 kg/m^3), and high values indicate high muscle stiffness (Koo et al., 2013). The mean value of two images at each time was used in the following analysis.

To evaluate intra-rater reliability of measurements, the intraclass correlation coefficient (1,2) ($ICC_{1,2}$) with

95% confidence interval (CI) was calculated from the shear elastic modulus of the two measurements at each time point. $ICC_{1,2}$ values were 0.985 (95% CI: 0.956-0.995) and 0.970 (95% CI: 0.912-0.990) at PRE and POST, respectively; therefore, good reliability was observed (Portney and Watkins, 2000).

2.4. Static stretching

The participants received SS in a prone position with the hip in a neutral position and the knee fully extended. The final angle was maintained for 30 s and SS was repeated at this angle for four bouts, corresponding to a total of 2-min SS. Immediately after each bout of SS, the ankle was manually moved to 20° plantar-flexion, at which the passive force of triceps surae would be almost zero (Hirata et al., 2015), in 3 s. In the R0 protocol, the ankle was immediately returned to the final angle in 3 s without being held at 20° plantar-flexion. In the R30 protocol, the ankle was held at 20° plantar-flexion for 30 s and then returned to the final angle in 3 s (Fig.3). The investigator carefully monitored these movements with a stopwatch to ensure that the ankle was moved in 3 s at each repetition.

2.5. Statistical analysis

Statistical analysis was performed via SPSS Statistics (version 22; IBM, Armonk, NY, USA). After confirming the normal distribution of each variable via the Shapiro–Wilk test, the following analyses were performed. The final angle, which was defined before each SS protocol, was compared via a paired t-test between the two protocols to examine whether the angle at which SS was performed differed between protocols.

With respect to the shear elastic modulus values, a two-way repeated measures ANOVA based on two factors [protocol (R0, R30) × time (PRE, POST)] was performed. When a significant interaction effect was obtained, a post-hoc test (paired t-test) was performed to examine the simple main effect of time. In addition, the change and the percentage change in shear elastic modulus were calculated as follows:

change in shear elastic modulus = PRE-value – POST-value

percentage change in shear elastic modulus = (change in shear elastic modulus/PRE-value) × 100

The change and the percentage change in shear elastic modulus were compared between two protocols via a paired t-test. The statistical significance was set at 5%.

3. Results

The EMG activities of lateral gastrocnemius were < 5% of maximal voluntary contraction in all participants, indicating that the muscle was almost inactive during SS and SWE measurements. The final angle corresponded to 37.7 ± 7.5° in R0 and 39.2 ± 6.9° in R30. The results of a paired t-test did not indicate a significant difference between protocols ($p = 0.289$), indicating that SS was performed at almost the same angle in the two protocols.

The shear elastic modulus values are listed in Table 1. The result of a two-way repeated measures ANOVA indicated a significant interaction effect ($p = 0.023$, $F = 6.56$, effect size = 0.319). Post-hoc paired t-tests comparing PRE and POST yielded uncorrected p-value of < 0.001 and 0.029 for R0 and R30 protocols, respectively. Additionally, both the change and the percentage change in shear elastic modulus in R0 exceeded that in R30 (p =

0.023 and 0.031, respectively).

4. Discussion

In this study we investigated the effect of four bouts of 30-s SS with different rest intervals (0 s, 30 s) on muscle stiffness of MG, and the results indicated that the decrease in muscle stiffness observed after SS with 0-s rest interval exceeded the decrease in stiffness after SS with 30-s of rest between stretching intervals. To the best of our knowledge, this is the first study that investigated the effect of rest interval duration between SS repetitions on muscle stiffness given a constant total stretching time. In addition, the muscle stiffness of MG decreased after both stretching protocols. This result is consistent with previous studies (Akagi and Takahashi, 2013; Kay et al., 2015).

The reason as to why the decrease in muscle stiffness was smaller in R30 when compared to that in R0 is potentially related to the degree of recovery in muscle stiffness during rest interval between each bout of SS. Stress relaxation is reported as a phenomena caused by SS, which corresponds to a gradual decline in the passive force on the muscle during stretching at a constant angle (Taylor et al., 1990). In a manner similar to the gradual declines in passive force during SS, it is known that the muscle force also gradually recovers after the muscle is released from stretching (Duong et al., 2001). Duong et al., (2001) reported that the decrease in force due to 20-min SS recovered 2 min after SS by approximately 43%. Another study reported that the decrease in MTU stiffness due to 5 bouts of 1-min SS returned to the baseline within 10 min after SS (Mizuno et al., 2013). In this study, SS with longer rest interval, such as that in R30, resulted in a smaller effect on the decrease in muscle stiffness owing to a certain amount of recovery between SS repetitions, although this recovery did not cancel the SS effect of each repetition.

There are a few limitations in the study. First, the study only investigated the acute effect of SS, and thus the prolonged or long-term effect is unclear. Second, all the participants were healthy young men. Future studies should investigate the influence of rest interval time in SS for different populations and/or in long-term intervention. Third, the shear elastic modulus was measured on one specific point of MG; therefore, these findings may not necessarily apply to the whole MG. Moreover, we focused only on muscle stiffness and not tendon stiffness. Finally, the effects on ROM or passive torque, that is, MTU stiffness remain unclear. This is because it was not possible to measure them immediately after SS owing to the time spent resetting the experimental setup.

In conclusion, the study investigated the effect of four bouts of 30-s SS with different rest intervals, namely 0 s and 30 s, on muscle stiffness of the medial gastrocnemius. The results indicated that SS with 0-s rest interval decreased muscle stiffness to a greater extent although SS with 30-s rest interval also decreased muscle stiffness. The results indicated that SS with a shorter rest interval may be more effective in decreasing muscle stiffness. Clinically, SS with shorter rest intervals could be recommended to decrease muscle stiffness from the viewpoint of injury prevention. Future studies are needed to investigate in greater detail the effect of rest interval duration between stretching repetitions to support our findings.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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Table

Table 1. Shear elastic modulus values of the medial gastrocnemius in each protocol

	Shear elastic modulus (kPa)				Percentage change in shear elastic modulus (%)	Interaction effect
	PRE	POST	p-value of post-hoc test	Change in shear elastic modulus		
R0	11.5 ± 3.3	10.0 ± 2.6 **	$p < 0.001$	1.6 ± 0.9 †	13.0 ± 5.2 †	$p = 0.023$, $F = 6.559$
R30	11.0 ± 2.8	10.2 ± 2.1 *	$p = 0.029$	0.8 ± 1.3	6.0 ± 10.4	effect size = 0.319

Values are expressed as mean ± standard deviation

PRE: before SS, POST: immediately after SS

R0: 0-s rest interval, R30: 30-s rest interval

Change in shear elastic modulus = PRE-value – POST-value

Percentage change in shear elastic modulus = (change in shear elastic modulus/PRE-value) × 100

* significant difference between time points ($p < 0.05$)

** significant difference between time points ($p < 0.01$)

† significant difference between protocols ($p < 0.05$)

Figure captions

Fig.1 Experimental setup

(a) Participant is in a prone position with the hip in neutral position and the knee fully extended. In determining the final angle, the participants pressed the remote button to stop the dynamometer.

(b) The foot on the right is attached securely to the footplate of the dynamometer. The axis of the ankle joint corresponds to the axis of the dynamometer rotation.

Fig.2 Typical example of shear wave elastography image

The region of interest (ROI) was set near the center of the muscle belly bulge of the medial gastrocnemius. A 10 mm circle was drawn at the center of the ROI. Shear elastic modulus was calculated from the mean shear wave speed in the circle.

Fig.3 Experimental protocol of stretching

Maximal dorsiflexion angle was defined prior to the stretching.

Static stretching for 30 s at the final angle was repeated for four bouts.

PF: plantar-flexion

Fig.1

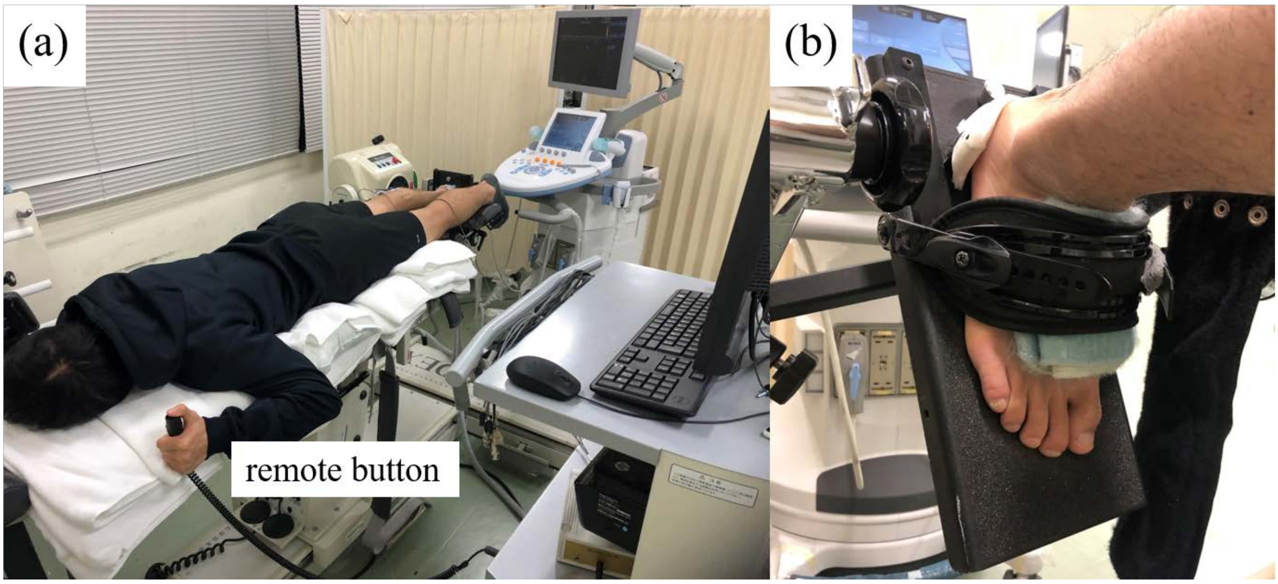
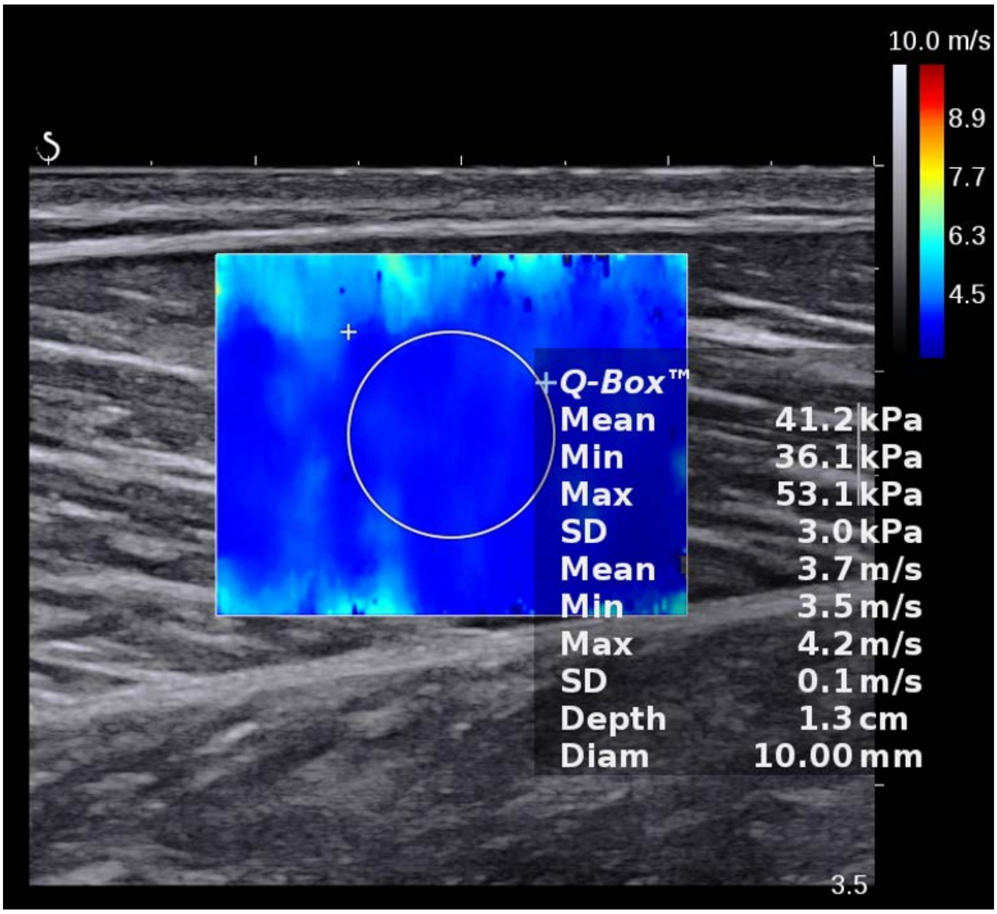
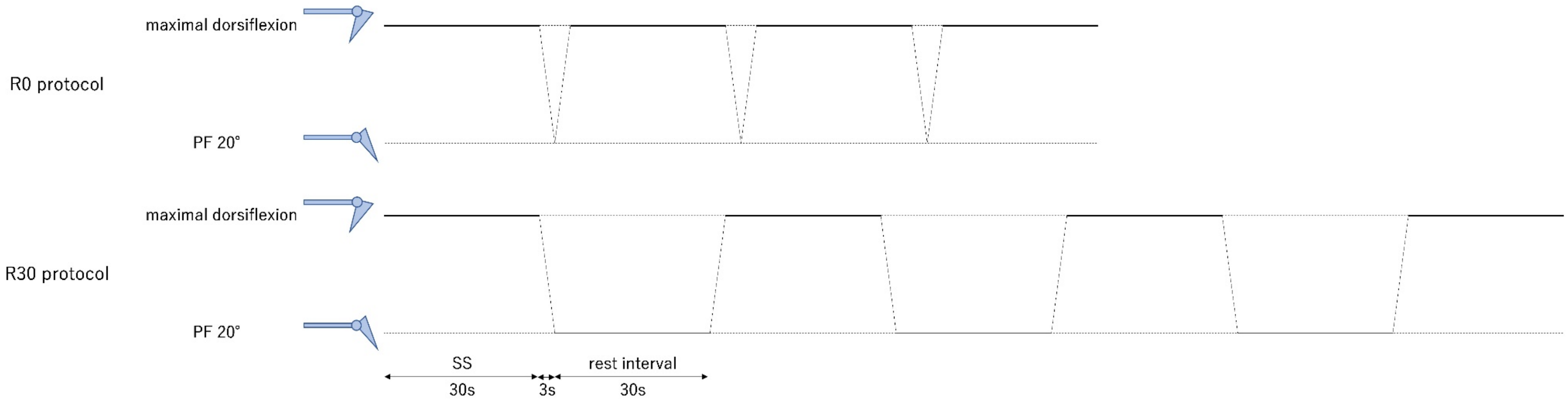

(a)
(b)
remote button

Fig.2


10.0 m/s
8.9
7.7
6.3
4.5
Q-Box™
Mean 41.2 kPa
Min 36.1 kPa
Max 53.1 kPa
SD 3.0 kPa
Mean 3.7 m/s
Min 3.5 m/s
Max 4.2 m/s
SD 0.1 m/s
Depth 1.3 cm
Diam 10.00 mm
3.5

302 Fig.3


maximal dorsiflexion
R0 protocol
PF 20°
maximal dorsiflexion
R30 protocol
PF 20°
SS
30s
3s
rest interval
30s

303